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(54) Title: COX-2 SELECTIVE INHIBITORS FOR MANAGING LABOUR AND UTERINE CONTRACTIONS

(57) Abstract

The present invention concerns a method of substantially preventing or reducing at least one of the changes in the female reproductive system associated with the onset or continuation of labour, the method comprising administering to the female an effective amount of a compound which selectively inhibits cyclo-oxygenase-2 (COX-2) function. Also provided is a method of substantially preventing or reducing uterine contractility either occurring during pregnancy or associated with menorrhagia. The method comprising administering to the female an effective amount of a compound which selectively inhibits cyclo-oxygenase-2 (COX-2) function. Preferably the compound is nimesulide, flosulide or meloxicam and can be used in combination with a progestogen, a progestin or a progestational agent.

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COX-2 SELECTIVE INHIBITORS FOR MANAGING LABOUR AND UTERINE CONTRACTIONS

The present invention relates to compounds for use in managing labour, in particular compounds related to the management of pre-term contractions.

Pre-term labour remains a significant problem in modern obstetric practice. It occurs in up to ten percent of all births but accounts for over 85% of perinatal deaths and an unknown proportion of mental and physical handicap (Martius and Eschenbach (1990) "The role of bacterial vaginosis as a cause of amniotic fluid infection, chorioamnionitis and prematurity - a review" *Arch. Gynecol. Obstet.* **247(1)**, 1-13. Although advances in neonatal intensive care have brought improvements in survival and morbidity rates for pre-term infants there has been little improvement in therapeutic options to stop pre-term contraction in the last decade.

There is evidence for the roles of prostaglandins in the early biochemistry of human parturition (Bennett (1988) "The Biochemical Mechanisms of Preterm Labour" in *Contemporary Obstetrics and Gynaecology* Ed. G.V.P. Chamberlain, Butterworth, London; Challis *et al* (1991) "Endocrinology of labour" *Fet. Med. Rev.* 3, 47-66). Prostaglandins are formed from the precursor arachidonic acid. Arachidonic acid is a substrate for at least three enzyme groups. Metabolism *via* the cyclo-oxygenase pathway produces the classical prostaglandins, prostacyclin and thromboxane. Metabolism *via* the lipoxygenase enzyme pathways produces a series of hydroxyeicosatetraenioc acids (HETEs) including the leukotrienes, and metabolism *via* epoxygenase pathways produces a series of epoxyeicosatetraenioc acids.

The principle sources of the prostaglandins which initiate labour are the

fetal membranes and decidua. Amnion contains large stores of arachidonic acid. Prior to labour arachidonic acid metabolism in amnion is principally *via* the lipoxygenase enzyme pathways to produce 5- and 12-HETE and leukotriene B4 (Bennett *et al* (1993) "Changes in arachidonic acid metabolism in amnion cells associated with increased cyclo-oxygenase gene expression at parturition" *Br. J. Obstet. Gynaecol.* **100**, 1037-42). There is also production of 14,15-epoxyeicosatetraenioc acid (Patel *et al* (1989) "Production of epoxygenase metabolite by human reproductive tissues" *Prostaglandins* **38(6)**, 615). In association with labour, there is an increase in overall arachidonic acid metabolism and a change in the ratio of cyclo-oxygenase to lipoxygenase metabolism to favour synthesis of prostaglandin E2. These changes are also seen with pre-term labour (Bennett *et al* (1987) "Preterm Labour: Stimulation of arachidonic acid metabolism in amnion cells by bacterial products" *Am. J. Obstet Gynecol.* **156**, 649-55). Prostaglandin E2 is known to mediate cervical ripening and to cause uterine contractions.

The roles of the lipoxygenase metabolites of arachidonic acid in amnion are unknown. Work performed by Bennett *et al* (1988) ("The effects of lipoxygenase metabolites of arachidonic acid upon contractility of human pregnant myometrium *in vitro*" *Prostaglandins* **33**, 837) and by Walsh (1989) ("5-Hydroxyeicosatetraenioc acid, leukotriene C4 and prostaglandin F2a in amniotic fluid before and during term and pre-term labor" *Am. J. Obstet. Gynecol.* **161**, 1352) has shown that 5-HETE is oxytocic and may play a role in prelabour (Braxton-Hicks) contractions. Its production, as part of an inflammatory reaction may also, therefore, play a role in the contractions of pre-term labour.

Whilst the central role of prostaglandins in the initiation of human labour is established, the mechanisms which control arachidonic acid metabolism

in the fetal membranes, and which stimulate prostaglandin production at term or pre-term are poorly understood. A wide range of theories have been proposed. It has been suggested that a fetal stimulus may act *via* the amniotic fluid. Candidates for the stimulus include platelet activating factor (PAF) (Hoffman *et al* (1990) "Detection of platelet activating factor in amniotic fluid of complicated pregnancies" *Am. J. Obstet. Gynecol.* **162**, 525-528) and interleukin 1 (IL1) (Romero *et al* (1988) "Infection and labor; Interleukin 1 a signal for the onset of parturition" *Am. J. Obstet. Gynecol.* **160**, 1117-1123; Romero *et al* (1990) "Amniotic fluid interleukin-1 in spontaneous labor at term" *J. Rep. Med.* **35(3)**, 235). Both of these are found in increased concentration in the amniotic fluid in association with labour, and both will stimulate prostaglandin production in amnion cells *in vitro*. PAF is believed to be a particularly strong candidate since its secretion by the fetus into the amniotic fluid is related to fetal lung maturity. In the sheep the onset of labour is associated with an increase in fetal cortisol secretion leading to increased placental oestradiol synthesis at the expense of progesterone. Such changes cannot be demonstrated in the maternal circulation in the human. An alternative hypothesis is an increase in the activity of the CRH/ACTH/cortisol system within the fetal membranes and placenta leading to local changes in steroid hormone concentrations which in turn stimulate increased prostaglandin synthesis (Challis *et al* (1991) "Endocrinology of labour" *Fed. Med. Rev.* **3**, 47-66).

Until recently it was phospholipase activity which was thought to be the limiting factor in prostaglandin production in the fetal membranes. However any stimulus to prostaglandin production must also increase the activity of the enzyme cyclo-oxygenase since it has a short half life and undergoes destruction after a limited number of reactions (Marshall *et al* (1979) "Constraints on prostaglandin synthesis in tissues" *J. Biol. Chem.*

262, 3510-3517)). Evidence that amnion produces only lipoxygenase metabolites of arachidonic acid before labour, and the change in the ratio of cyclo-oxygenase:lipoxygenase metabolism with labour suggests that the activities of the two enzymes, COX and phospholipase, must be independently but coordinately controlled (Bennett *et al* (1987) "Preterm Labour: Stimulation of arachidonic acid metabolism in amnion cells by bacterial products" *Am. J. Obstet. Gynecol.* **156**, 649-55; Bennett *et al* (1993) "Changes in arachidonic acid metabolism in amnion cells associated with increased cyclo-oxygenase gene expression at parturition" *Br. J. Obstet. Gynaecol.* **100**, 1037-42). The enzyme kinetics studies of Smieja *et al* (1993) "Prostaglandin endoperoxide synthase kinetics in human amnion before and after labour at term and following pre-term labour" *Placenta* **14**, 163-175) suggest that the increase in COX activity in amnion with the onset of labour is due entirely to an increase in the synthesis of the COX enzyme.

A cyclo-oxygenase gene which produces a mRNA transcript of 2.8 kb was reported in human and other species in 1988 and 1989 (DeWitt and Smith (1988) "Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complimentary DNA sequence" *Proc. Natl. Acad. Sci. USA* **85**, 1412-1416; Merlie *et al* (1988) *J. Biol. Chem.* **263(8)**, 3550-3553; Yokoyama and Tanabe (1989) "Cloning of the human gene encoding prostaglandin endoperoxide synthase and primary structure of the enzyme" *Biochem. Biophys. Res. Comm.* **165(2)**, 888-894). In 1991 a second cyclo-oxygenase gene was reported whose expression is induced by mitogens and inhibited by glucocorticoids (O'Banion *et al* (1991) "A serum and glucocorticoid regulated 4 kb mRNA encodes a cyclo-oxygenase related protein" *J. Biol. Chem.* **266(34)**, 23261-23267). This gene usually encodes a mRNA of greater than 4 kb. The transcript size difference between the type 1 and type 2 cyclo-oxygenases is

accounted for by a long 5′ untranslated region in the type 2 mRNA. Although not identical, the type 1 and type 2 proteins are of similar molecular size and show a high degree of homology. At present there is no evidence that these two enzymes have any intrinsic functional differences.

The COX-1 and COX-2 genes are on different chromosomes but have a similar intron/exon arrangement. The COX-1 gene spans over 22 kb of genomic DNA whilst the COX-2 gene spans only 8 kb. The long 3′ untranslated portion of COX-2 mRNA contains multiple copies of the Shaw-Kamen sequence (AUUUA) which are a feature of early response genes with rapid mRNA degradation (Kosaska *et al* (1994) *Eur. J Biochem.* **221**, 889-97). Although not identical, the type 1 and type 2 proteins are of similar molecular size and show a high degree of homology. COX-1 appears to be constitutively expressed in cells with constant prostaglandin synthesis. COX-2 is an inducible, early response gene which mediates acute prostaglandin synthesis, for example in inflammation (Vane (1994) "Towards a better aspirin" *Nature* **367**, 215).

Slater *et al* (1994) "Implication of RNA binding proteins in the regulation of cyclo-oxygenase in human amnion at term" *Biochem. Biophys. Res. Comm.* **203(1)**, 67-71 describes experiments showing that a protein binds to COX-2 mRNA which may be involved in translation initiation or in mRNA stability.

We have found that northern blot analysis is unsatisfactory in the study of COX expression because of the low abundance of COX-1 and the homology between COX-1 and COX-2 cDNAs. We have therefore developed a reverse transcriptase polymerase chain reaction assay (RT-PCR) which allows the expression of the two genes to be independently

studied. We found that, in amnion, both before and after labour, the COX-2 mRNA has over 100 fold greater abundance than the COX-1 mRNA (Slater *et al* (1994) "The relative abundance of type 1 to type 2 cyclo-oxygenase mRNA in human amnion at term" *Biochem. Biophys. Res. Comm.* **198(1)**, 304-308). There appears to be no increase in COX-1 expression with labour but COX-2 transcription appears to double (Slater *et al* (1995) "Changes in the expression of types 1 and 2 cyclo-oxygenase in human fetal membranes at term" *Am. J. Obstet. Gynecol.* **172**, 77-82).

In a model of pre-term labour due to infection we have previously shown (Bennett *et al* (1987) "Preterm Labour: Stimulation of arachidonic acid metabolism in amnion cells by bacterial products" *Am. J. Obstet. Gynecol.* **156**, 649-55; Bennett *et al* (1993) *Br. J. Obstet. Gynaecol.* **100**, 1037-42) that bacteria may release phospholipases which increase arachidonic acid availability for synthesis to prostaglandins. Whether pre-term labour is due to infection or not there appears to be an associated inflammatory reaction with increased synthesis of cytokines such as interleukin 1β (IL-1β) within the uterus (Romero *et al* (1989) *Am. J. Obstet. Gynecol.* **160**, 1117-1123).

There are currently no drugs available which will safely and effectively inhibit pre-term contractions. The most commonly used agents, β-sympathomemetics such as Ritodrine, Salbutamol and Terbutaline, cause significant maternal cardiovascular, respiratory and metabolic side effects and may lead to pulmonary oedema, cardiac failure and maternal death. Furthermore they are subject to tachyphylaxis and become ineffective after 24 to 48 hours. This tachyphylaxis is probably due to down regulation, by β-sympathomemetics, of their own receptor. Meta-analysis of randomised controlled trials has shown that the value of β-sympathomemetics is only in the temporary delay of labour to allow *in*

utero transfer or administration of steroid to improve fetal lung surfactant production.

Indomethacin, a cyclo-oxygenase inhibitor is effective in preventing the contractions of pre-term labour. It is more effective in short term prolongation of pregnancy than the β-sympathomimetics and, unlike β-sympathomemetics, it can reduce the risk of delivery pre-term (Keirse (1995) "Indomethacin tocolysis in preterm labour" in *Pregnancy and Childbirth Module* (Eds. Enkin, M.W., Keirse, M.J.N.C., Renfrew, M.J., Neilson, J.P.) Cochrane Database of Systematic Reviews, No 04383, Oxford). The use of Indomethacin is limited by fetal side effects. Indomethacin reduces fetal urine output, both by reducing renal blood flow and by reducing prostaglandin E mediated inhibition of arginine vasopressin (AVP) in the collecting duct (Kirshon *et al* (1991) *"Long term indomethacin therapy decreases fetal urine output and causes oligohydramnios"* Am. J. Perinatol. **8(2)**, 86; Kosaska, T., Miyata, A., Ihara, S. *et al* (1994) "Characterisation of the human gene encoding prostaglandin endoperoxide synthase" *Eur. J. Biochem.* **221**, 889-97; Stevenson and Lumbers (1992) "Effects of indomethacin on fetal renal function, renal and umbilicoplacental blood flow and lung liquid production" *Journal of Developmental Physiology* **17**, 257-64; Walker *et al* (1994) "Indomethacin and arginine vasopressin interaction in the fetal kidney: A mechanism of oliguria" *American Journal of Obstetrics and Gynecology* **171**, 1234-41). Indomethacin also causes constriction of the ductus arteriosus (Moise *et al* (1995) *Am. J. Obstet. Gynecol.* **170(45)**, 1204-5), presumably mediated through suppressed synthesis of vasodilatory prostaglandins produced by the ductus. Although Doppler studies show an effect in all exposed fetuses, clinically significant ductal constriction occurs only in a proportion, increasing with gestational age from 10% at 26 weeks to 50% at 32 weeks. Accordingly the use of

indomethacin is limited in clinical practice to use ≤ 32 weeks, and to short courses (≤ 48 hours) after which any effects on the constriction of the ductus have been shown to be reversible (Tulzer *et al* (1991) "Doppler echocardiography of fetal ductus arteriosus constriction versus increased right ventricular output" *JACC* **18(2)**, 532-36; Moise *et al* (1993) "Effect of advancing gestational age on the frequency of fetal ductal constriction in association with maternal indomethacin use" *Am. J. Obstet. Gynecol.* **170(45)**, 1204-5; Respondek *et al* (1995) "Fetal echocardiography during indomethacin treatment" *Ultrasound Obstet. Gynecol.* **5**, 86-89). Short term use ≤ 32 weeks has not been associated with the complications of longer term therapy such as oligohydramnios and rarely premature closure of the ductus leading to pulmonary hypertension which may lead to persistance of the fetal circulation in neonatal life. Because of these side effects some obstetricians now use Sulindac, which appears to be equally good as a tocolytic (Carlon *et al* (1992) *Obstet. Gynecol.* **85(5)**, 769-774) in place of Indomethacin. Sulindac produces a smaller reduction in fetal urine output and minimal effect on ductal patency (Carlon *et al* (1992) *Obstet. Gynecol.* **85(5)**, 769-774; Rasanen and Jouppila (1995) "Fetal cardiac function and ductus arteriosus during indomethacin and sulindac therapy for threatened preterm labour; A randomised study" *Am. J. Obstet. Gynecol.* **173(1)**, 20-25). The reason for the differential effects is not known, but would appear related to its administration as a pro-drug, leading to less of the active drug crossing the placenta, and possibly less of the activating enzyme being present in ductal tissue (Kramer *et al* (1995) "Placental transfer of sulindac and its active metabolite in humans" *Am. J. Obstet. Gynecol.* **172(3)**, 886-90). However, Sulindac is far from an ideal choice of tocolytic agent.

Nimesulide is a non-steroidal, anti-inflammatory drug (NSAID) which is widely prescribed in Europe for the management of connective tissue

inflammatory disorders, post operative analgesia and febrile episodes in children. It is comparable in efficacy to other NSAID's but has fewer gastric side effects. Previous studies of its mechanism of action have suggested that, unlike other NSAID's, it does not inhibit the action of prostaglandin endoperoxide synthase (PGHS, cyclo-oxygenase) or the synthesis of prostaglandins (Magni (1993) "The effect of Nimesulide on prostanoid formation" *Drugs* **46 (Suppl. 1)**, 10-14.

There is therefore a need for improved methods of managing pre-term labour and, in particular, pre-term contractions. It is an object of the invention to provide such improvements.

A first aspect of the invention provides a method of substantially preventing or reducing at least one of the changes in the female reproductive system associated with the onset or continuation of labour the method comprising administering to the female an effective amount of a compound which selectively inhibits cyclo-oxygenase-2 (COX-2) function.

Cyclo-oxygenase-2 (COX-2) is also called prostaglandin endoperoxide synthase-2 (PGHS-2). The COX-2 gene and the sequence of its polypeptide product are described in O'Banion *et al* (1991) *J. Biol. Chem.* **266**, 23261-23267 incorporated herein by reference.

During the onset of labour the female reproductive system (which includes the uterus, cervix and vagina) undergoes various biochemical changes which prepare the female for delivery.

For example, the uterus increases in contractility and undergoes contractions. The cervix also ripens in readiness for delivery. Such changes are well known in the arts of obstetrics, gynaecology and

midwifery and, for example, the Bishop's score indicates the degree of cervical ripening.

There are many situations where it is useful to substantially prevent or reduce at least one of the changes in the female reproductive system associated with the onset or continuation of labour. For example, it is well known that certain groups of pregnant women are at high risk of pre-term labour. Women that have had one or more pre-term labours previously are at considerably higher risk of a further pre-term labour when pregnant. An increased risk of pre-term labour can also be determined by measuring oncofoetal fibronectin levels and by cervical examination using methods well known in the art.

Various infections, especially bacterial infections, are also known to increase the risk of pre-term labour.

Thus, the method of the invention is particularly useful as a prophylactic method in those pregnant women at risk of pre-term labour.

It is also useful to prevent or reduce at least one of the changes in the female reproductive system associated with the continuation of labour, particularly uterine contractions, temporarily in circumstances where this is desirable. For example, it may be desirable temporarily to inhibit uterine contractions during labour in order to clear the foetal lungs or in order to transfer the woman from one place to another. It is often desirable to transfer the woman to a more suitable place where better care is available for her and the baby.

Thus, the method of the invention allows for the interruption of labour by inhibiting uterine contractions.

It is also useful to substantially prevent for a considerable duration pre-term labour using the method of the invention. In particular, it is useful to inhibit pre-term uterine contractions from the time when they first occur (or soon thereafter) until the normal time of delivery. Thus, typically, the woman is administered an effective amount of a compound which selectively inhibits COX-2 function from the time she presents with pre-term contractions to between 37 and 42 weeks following conception.

A particularly preferred embodiment of the invention therefore provides a method of substantially preventing or reducing uterine contractility or uterine contractions associated with pre-term labour the method comprising administering to the female an effective amount of a compound which selectively inhibits COX-2 function.

Thus, a compound which selectively inhibits COX-2 function substantially reduces or prevents uterine contractions. Contractility is the rate or extent of contraction; the uterus has intrinsic contractility.

Thus, the compound which selectively inhibits COX-2 function acts as a tocolytic agent.

A further particularly preferred embodiment provides a method of substantially preventing or reducing ripening of the cervix the method comprising administering to the female an effective amount of a compound which selectively inhibits COX-2 function.

A second aspect of the invention provides a method of substantially preventing or reducing uterine contractility the method comprising administering to the female an effective amount of a compound which selectively inhibits COX-2 function. As for the first aspect of the

invention the uterine contractility (or uterine contractions) may be associated with the onset or continuation of labour, especially the onset of pre-term labour.

Suitably, the female is pregnant and the uterine contractility occurs during pregnancy. Conveniently, the method substantially prevents or reduces pre-term labour.

However, the method of the second aspect of the invention is also useful in substantially preventing or reducing uterine contractility or contractions in non-pregnant women. Such uterine contractions occur in some women during menorrhagia (excessive uterine bleeding occurring at regular intervals of menstruation). Thus, the invention includes the substantial prevention or reduction in menorrhagia in women by administration of a compound which selectively inhibits COX-2 function.

It is preferred if the compound is administered following (a) signs of pre-term labour in the pregnant woman, or (b) an indication that the pregnant woman is at risk of pre-term labour, especially uterine contractions, or (c) symptoms of menorrhagia.

It is preferred if administration of the compound commences during the second or third trimester of pregnancy when the compound is administered prophylactically.

If the compound is used to substantially inhibit contractions during pre-term labour it is preferred if the compound is administered for a period of between 24 and 72 hours, preferably 48 hours.

The compound, or a formulation thereof, may be administered in any

conventional way. The compound, or a formulation thereof, may be administered intravenously or via intraamniotic or intravaginal administration but it is particularly preferred if the compound or formulation is administered orally or rectally to the mother. The treatment may consist of a single dose or a plurality of dose over a period of time.

The compound may selectively inhibit COX-2 function at any level. Suitably, the compound selectively inhibits COX-2 enzyme activity.

By "selectively inhibits COX-2 enzyme activity" we mean that the compound preferably inhibits COX-2 in preference to other cyclo-oxygenase enzymes, in particular in preference to cyclo-oxygenase-1 (COX-1). The COX-1 gene and the sequence of its polypeptide product are described in Yokoyama and Tanabe (1989) *Biochem. Biophys. Res. Comm.* **165**, 888-894 incorporated herein by reference. COX-2 is also called PGHS-1.

Conveniently, the compound which selectively inhibits COX-2 enzyme activity is at least ten times better at inhibiting COX-2 than COX-1; preferably it is at least fifty times better; preferably it is at least one hundred times better; still more preferably it is at least one thousand times better and in greater preference it is at least ten thousand times better.

It is most preferred if the compound has substantially no inhibitory activity against the COX-1 enzyme.

Conveniently, the compound selectively inhibits COX-2 enzyme production. The compound may, for example, selectively prevent transcription of the COX-2 or it may selectively prevent translation of the

COX-2 message.

By "selectively inhibits COX-2 enzyme production" we mean that the compound preferably inhibits the production of COX-2 in preference to other cyclo-oxygenases, in particular in preference to the production of COX-1.

Conveniently, the compound which selectively inhibits COX-2 enzyme production is at least ten times better at inhibiting COX-2 production than COX-1 production; preferably it is at least fifty times better; more preferably it is at least one hundred times better; more preferably still it is at least one thousand times better; and in greater preference it is at least ten thousand times better.

It is most preferred if the compound has substantially no inhibitory activity against COX-1 enzyme production.

Methods for identifying whether a particular molecule is a compound which selectively inhibits COX-2 function include the following:

Activated macrophages are known to express COX-2 and thereby synthesise prostaglandin. Seminal vesicle cells are known to express COX-1 and thereby synthesise prostaglandin. Compounds which selectively inhibit prostaglandin synthesis in activated macrophages compared to prostaglandin synthesis in seminal vesicle cells are compounds which selectively inhibit COX-2 function. By measuring the synthesis of prostaglandins from their precursor arachidonic acid automated screening procedures can readily be devised by the person skilled in the art. It will be appreciated that the compounds identified by this screen may be COX-2 enzyme inhibitors or compounds which

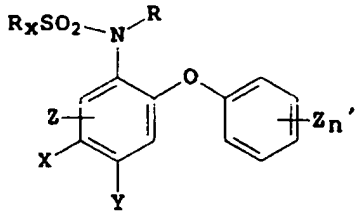
selectively inhibit COX-2 enzyme production, for example by inhibiting COX-2 gene transcription or COX-2 mRNA translation.

Alternatively or additionally cells which produce COX-1 or COX-2, and can be used in a cell-based assay can be made by transfection with the relevant gene or cDNA. The COX-1 and COX-2 cDNAs are known as disclosed above.

Pure COX-1 and COX-2 enzyme can be produced using recombinant DNA techniques and the crystal structures of COX-1 and COX-2 are known. Biochemical screening of test compounds to select COX-2 selectively inhibitors can be carried out using purified COX-1 and COX-2 enzyme using methods that are known to the person skilled in the art.

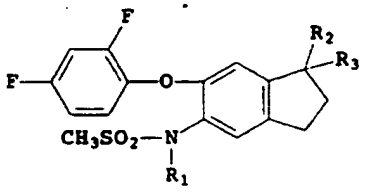
The method described in WO 95/18969, incorporated herein by reference, may be useful in identifying selective inhibitors of COX-2 function. Test compounds for screening by any suitable method may be from any library, or collection, of chemicals, including those made by combinatorial chemistry and derived from plant extracts. By "chemicals" we include molecules such as oligonucleotides and the like.

A suitable collection of chemicals to screen are those described in US 3,840,597 to Moore and Harrington, incorporated herein by reference, especially those compounds with a formula



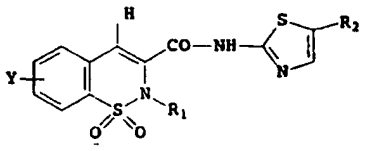
wherein R_x is an optionally halogenated alkyl radical, R is hydrogen, alkyl or a pharmaceutically acceptable cation, X is alkoxy, alkyl, halogen, acetamido, nitro, hydrogen, amino, alkoxycarbamoyl or dialkylamino, Y is nitro, amino, alkoxycarbamoyl, dialkylamino or hydrogen, provided that one of X and Y is nitro, amino, alkoxycarbamoyl, or dialkylamino, Z is halogen, nitro or hydrogen, Z' is halogen, alkyl, alkoxy, nitro, amino, alkanamido, haloalkyl, hydroxy, dialkylamino, alkoxycarbamoyl, alkylthio, alkylsulfonyl, alkanoyl, or alkylsulfinyl and *n* is 0-2, provided that the individual aliphatic groups appearing to the R_x, R, X, Y, and Z' moieties contain from one to four carbon atoms each, but excluding nimesulide.

Also suitable as a collection of chemicals to screen are those described in GB 2 092 144 to Schering, incorporated herein by reference, especially those compounds with a formula



in which R_1 represents a hydrogen atom, a methanesulphonyl group or an acetyl group, and R_2 and R_3 together represent an oxo group or an oximino group or R_2 represents a hydrogen atom and R_3 represents a hydrogen atom, a hydroxyl group or an amino group, but excluding flosulide.

Also suitable as a collection of chemicals to screen are those described in US 4,233,299 to Engel *et al*, incorporated herein by reference, especially those compounds with a formula



wherein R_1 is hydrogen, methyl or ethyl; R_2 is methyl, ethyl or n-propyl; and Y is hydrogen, methyl, methoxy, fluorine or chlorine or a non-toxic, pharmacologically acceptable salt thereof formed with an inorganic or organic base, but excluding meloxicam.

The synthesis of at least some of the above-mentioned collections of chemicals is described in the relevant aforementioned patent or patent application.

Inclusion complexes of nimesulide alkali and alkaline earth salts may also be useful such as those described by WO 94/28031, incorporated herein by reference.

A particularly preferred embodiment is wherein the compound is any one of nimesulide, 4-hydroxynimesulide, flosulide, and meloxicam.

Nimesulide is N-(4-nitro-2-phenoxyphenyl) methanesulfonamide (also called 4-nitro-2-phenoxymethanesulfonanilide). Nimesulide is 100-fold more specific for COX-2 inhibition than for COX-1 inhibition. Nimesulide is manufactured by Boehringer.

Flosulide is 6-(2,4-difluorophenoxy)-5-methyl sulphonylamino-1-indanone (also known as N-6-(2,4-difluorophenoxy)-1-oxo-indan-5-yl methane-sulphonamide). Flosulide is 1000-fold more specific for COX-2 inhibition than for COX-1 inhibition. Flosulide is manufactured by Ciba Geigy.

Meloxicam is 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide. Meloxicam is 1000-fold more specific for COX-2 inhibition than for COX-1 inhibition. Meloxicam is manufactured by Boehringer.

The synthesis of nimesulide is well known and is described in US 3,840,597; the synthesis of flosulide is well known and is described in GB 2 092 144; and the synthesis of meloxicam is well known and is described in US 4,233,299.

Other COX-2-specific inhibitors which may be useful in the practice of the invention include:

L 475 L337 which is 500-fold more specific for COX-2 inhibition than for COX-1 inhibition. This is manufactured by Merck Frost.

SC 58125 Celecoxib which is 100-fold more specific for COX-2 inhibition than for COX-1 inhibition. Celecoxib is manufactured by Searle.

NS 398 which is manufactured by Taisho and which is very highly selective for COX-2.

DuP 697, which is COX-2-selective and is manufactured by DuPont.

Preferably, the female is administered between 0.1 and 40 mg/kg of nimesulide; more preferably between 1 and 20 mg/kg; still more preferably between 2 and 10 mg/kg; and most preferably between 3 and 6 mg/kg.

Preferably, the female is administered between 0.1 and 40 mg/kg of

flosulide; more preferably between 1 and 20 mg/kg; still more preferably between 2 and 10 mg/kg and most preferably between 3 and 6 mg/kg.

The amount of nimesulide, flosulide or meloxicam administered in the methods of the present invention may be the same as the amount administered in other indications for these drugs. Conveniently, in order to prevent uterine contractions, 200 mg of nimesulide may be administered to the woman every twelve hours for four doses, or it may be administered as a 200 mg suppository once daily.

Nimesulide, flosulide and meloxicam are COX-2 enzyme inhibitors, probably competitive inhibitors.

The female may be any female mammal such as human, horse, pig, cow, sheep, dog and cat.

It is preferred if the female is a human female.

A particular advantage of the present invention is that the COX-2 inhibitor reduces the possibility of harm being done to the foetus compared with other tocolytic agents. This is particularly the case when the COX-2 inhibitor is nimesulide, flosulide or meloxicam; more particularly when it is nimesulide.

A third aspect of the invention provides a pharmaceutical composition comprising a compound which selectively inhibits COX-2 function and a further component that is usefully administered to a female who has or is at risk of pre-term labour, or a female who has or is at risk of uterine contractions, or a female who suffers from menorrhagia.

Thus, the pharmaceutical composition is useful in the methods of the first or second aspects of the invention. The said further compound that is usefully administered may be any compound that is useful to administer with the compound which selectively inhibits COX-2 enzyme function in the first or second aspects of the invention.

It is preferred if the further compound is a progestogen, progestin or other progestational agent. In particular is included the naturally occurring hormone progesterone and its analogues such as allyloestrenol, dydrogesterone, hydroxyprogesterone and medroxyprogesterone. Progestogens also include testosterone analogues such as norethisterone.

Progesterone is preferred.

The further compound may be a tocolytic and conveniently is any of beta-sympathomimetics, anti-oxytocins or calcium channel blockers as well as progesterone.

A fourth aspect of the invention provides a pharmaceutical composition comprising a compound which selectively inhibits COX-2 function wherein the composition is in a form adapted for delivery to the female reproductive system.

By "female reproductive system" we include those parts that develop during pregnancy including the amnion.

Preferably, the said composition is in a form adapted for delivery *via* or into the rectum, vagina or amnion of the mother; also preferably the composition is adapted for delivery to the cervix. Thus, conveniently the composition is a pessary, sponge, ring, gel or other device adapted for

delivery to the female reproductive system, particularly the vagina. Pessaries, gels, sponges, rings and other such devices are well known in the art, for example in various chemical contraceptive methods.

It is well known that the pH of the vagina is usually acid due to the presence of Lactobacilli; it is preferred if the said compound is prepared in a composition in a form which is relatively stable to acidic conditions, especially those found in the vagina.

It is also preferred if the composition is compatible with the amniotic fluid and that the composition is delivered into the amnion. The amniotic fluid has a distinct pH and a distinct osmotic tension. It is particularly preferred if the composition comprises a compound which selectively inhibits COX-2 function and a further component, such as a liquid in which said compound is dispersed or dissolved, which has substantially the same pH or substantially the same osmotic tension as amniotic fluid. The amniotic fluid pH and osmotic tension are well known to, or can be readily measured by, the person skilled in the art.

It is preferred if the compound selectively inhibits COX-2 enzyme activity.

It is also preferred if the compound selectively inhibits COX-2 enzyme production.

It is particularly preferred if the compound is any one of nimesulide, flosulide, 4-hydroxynimesulide, or meloxicam.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the

active ingredient (for example, COX-2-selective inhibitor) with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (eg sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide desired release profile.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually

sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

A fifth aspect of the invention provides use of a compound which selectively inhibits COX-2 function in the manufacture of a medicament for substantially preventing or reducing uterine contractility, or for

substantially preventing or reducing at least one of the changes in the female reproductive system associated with the onset or continuation of labour.

A sixth aspect of the invention provides use of a compound which selectively inhibits COX-2 function as a tocolytic agent.

The invention will now be described in more detail with reference to the following Examples and Figures wherein

Figure 1 shows ethidium stained gel showing COX-2 RT-PCR products amplified from fetal membranes at various gestational ages.

Figure 2 shows the effect of IL-1β (top panel) and TPA (lower panel) upon expression of COX-2 (measured by qRT-PCR) in WISH cells.

Figure 3 shows the effect of LPS upon expression of COX-2 (measured by qRT-PCR) in intact fetal membranes.

Figure 4 shows ethidium stained gels showing expression of COX-1 (lower panel) and COX-2 (upper panel) in human fetal heart at between 20 and 24 weeks.

Figure 5 shows the action of nimesulide on prostanoid formation in human fetal membranes stimulated with interleukin-1β.

Figure 6 shows the effect of prophylactic Nimesulide upon amniotic fluid index in a single patient at very high risk of preterm labour. Nimesulide was given from 17 to 34 weeks. Preterm labour began 7 days after therapy was stopped. Amniotic fluid index remained within normal limits

throughout treatment whereas treatment with the COX non-selective drug Indomethacin characteristically causes oligohydramnios within 7 days.

Figure 7 shows the effect of Nimesulide upon contractility in isolated human pregnant myometrial strip in an organ bath. Nimesulide significantly inhibits uterine contractions, suggesting an important role for COX-2 in uterine contractility.

Figure 8 shows COX-1 (top panel) and COX-2 (bottom panel) expression in myometrium at various gestational ages and before and after labour. Expression of the two genes is at similar levels of mRNA abundance but COX-2 expression increases near to term and with labour. This shows that increased prostaglandin synthesis in myometrium associated with labour is due to COX-2. It is likely that COX-1 mediates the synthesis of prostacyclin, a prostaglandin that inhibits uterine contractility, whereas COX-2 mediates synthesis of prostaglandin F2a which stimulates contractions. Use of a COX-2 specific antiprostaglandin in preterm labour would inhibit prostaglandin F2a synthesis but not prostacyclin synthesis whereas a non-selective antiprostaglandin would inhibit synthesis of both compounds.

Figure 9 shows the effect of Nimesulide upon PG synthesis in human fetal membranes compared with that of Indomethacin. Membranes have been stimulated using IL1b to upregulate PG synthesis. IL1b is thought to be an important mediator of preterm labour. Nimesulide shows a 100 fold selectivity for COX-2 whereas Indomethacin is non-selective. Both have similar effects upon membrane prostaglandin synthesis suggesting that IL-1b stimulation of prostaglandin synthesis in fetal membranes is mediated via COX-2 and not COX-1.

Example 1: COX-2 expression in pregnancy

When studied over a range of gestational ages (using fetal membranes obtained following pregnancy termination or pre-term caesarean section) we have found that there is a marked increase in COX-2 expression in the later part of the third trimester, before the onset of labour (Fig 1). It is believed that this increase in COX-2 expression produces the prostaglandins which mediate the remodelling of the cervix and explains why only a modest rise in COX-2 expression in fetal membranes collected at term before and after labour.

In experiments using an immortalised amnion cells line (WISH) we found that both IL-1β and the phorbol ester tumor promoting agent TPA caused a rapid induction of COX-2 expression with peak expression by 30 minutes (Fig 2). Similarly we have found that Lipopolysaccaride (LPS, a bacterial cell wall component) stimulation of human fetal membranes, in a model of infection, causes a biphasic increase in COX-2 expression, with peaks at 30 minutes and 8 hours, probably due to an initial direct effect on transcription followed by a secondary effect perhaps mediated by synthesis and release of cytokines such as IL-1β (Fig 3). These observations may explain the stimulation of both substrate release (*via* phospholipase) and prostaglandin synthesis (*via* cyclo-oxygenase) where pre-term labour is caused by infection. It also suggests that the increase in cyclo-oxygenase activity in pre-term labour is due to increased expression of COX-2. The biochemical mechanisms which act where preterm labour is not associated with infection are not currently known but our data predict that they will also involve increased expression of COX-2.

In the myometrium prior to labour the principal COX metabolite of

arachidonic acid is prostacylin. This acts to suppress myometrial contractility. With the onset of labour there is synthesis of prostaglandin F2a which is a powerful oxytocic. We believe that, in myometrium, prostacyclin synthesis is COX-1 mediated, whilst prostaglandin F2a synthesis is COX-2 mediated.

In contrast, we have shown that in fetal tissues such as heart, kidney, brain and gut, COX-1 expression exceeds that of COX-2 (Fig 4). This strongly suggests that it is COX-2 whose increased expression mediates the onset of labour at term whilst COX-1 mediates normal fetal physiological function such as vascular tone, ductal patency, glomerular filtration rate and tubular reabsorbation.

Example 2: Inhibition of COX-2 mediated prostaglandin synthesis by Nimesulide

We have used intact human fetal membranes, stimulated with interleukin-1β, to investigate the inhibition of COX-2 mediated prostaglandin synthesis by Nimesulide.

Two cm discs of fetal membranes obtained after elective caesarean section were cultured in medium containing Interleukin-1β (1 and 10 ng/ml) and Nimesulide (10 and 100 μM). Control wells contained culture medium without IL-1β or Nimesulide. After 24 hours Prostaglandin E2 concentration was measured by ELISA (Amersham Life Science). Stimulation with IL-1β at either concentration caused an increase in prostaglandin synthesis of almost two fold compared with basal production. Inhibition of prostaglandin production was profound and similarly effective at 10 and 100 μM Nimesulide. Significant inhibition ($p<0.05$) was apparent when comparing all Nimesulide groups with

control and cytokine induced control (Fig 5).

Previous studies which have suggested that Nimesulide does not exert its anti-inflammatory effects through inhibition of COX or prostaglandin synthesis used experimental systems based upon bovine seminal vesicle microsomes or gastric tissue, which express COX-1 (Magni (1993) *Drugs* **46 (Suppl 1)**, 10-14). These data can therefore be reinterpreted as showing that Nimesulide has little effect upon the constitutively expressed type-1 COX. In our system, which expresses COX-2, Nimesulide acts as a powerful antiprostaglandin. We have found that expression of COX-2 in fetal membranes is increased several weeks prior to onset of labour. This is believed to contribute to increased basal synthesis of prostaglandins which explains inhibition by Nimesulide to below levels of basal production in these experiments.

Although they are highly effective at inhibition of contractions and delaying of delivery (Keirse 1995) the use of anti-prostaglandins such as Indomethacin in pre-term labour is limited by fetal side effects. These include constriction of the ductus arteriosis, oligohydramnios, renal tubular dysfunction, intracranial haemorrhage and necrotising entercolitis. It is likely that each of these side effects is mediated by inhibition of constitutively synthesised prostaglandins in fetal tissues. Since constitutive prostaglandin synthesis is likely to be mediated by COX-1, the use of at least some COX-2 specific anti-prostaglandins allows inhibition of pre-term contractions without fetal side effects.

Figure 7 shows the effect of Nimesulide upon contractility in isolated human pregnant myometrial strip in an organ bath. Nimesulide significantly inhibits uterine contractions, suggesting an important role for COX-2 in uterine contractility.

Figure 8 shows COX-1 (top panel) and COX-2 (bottom panel) expression in myometrium at various gestational ages and before and after labour. Expression of the two genes is at similar levels of mRNA abundance but COX-2 expression increases near to term and with labour. This shows that increased prostaglandin synthesis in myometrium associated with labour is due to COX-2. It is likely that COX-1 mediates the synthesis of prostacyclin, a prostaglandin that inhibits uterine contractility, whereas COX-2 mediates synthesis of prostaglandin F2a which stimulates contractions. Use of a COX-2 specific antiprostaglandin in preterm labour would inhibit prostaglandin F2a synthesis but not prostacyclin synthesis whereas a non-selective antiprostaglandin would inhibit synthesis of both compounds.

Figure 9 shows the effect of Nimesulide upon PG synthesis in human fetal membranes compared with that of Indomethacin. Membranes have been stimulated using IL1b to upregulate PG synthesis. IL1b is thought to be an important mediator of preterm labour. Nimesulide shows a 100 fold selectivity for COX-2 whereas Indomethacin is non-selective. Both have similar effects upon membrane prostaglandin synthesis suggesting that IL-1b stimulation of prostaglandin synthesis in fetal membranes is mediated via COX-2 and not COX-1.

Example 3: Patient treated with Nimesulide

The patient was a 31 year old in her tenth pregnancy with no live children. Eight previous pregnancies, managed abroad, had ended in spontaneous preterm delivery at 27, 27, 26, 25, 29, 30, 27 and 32 weeks with early neonatal death in every case. She had also had one spontaneous 18 week miscarriage. Vaginal cervical circlage was performed in the fourth and subsequent pregnancies. Prior to this

pregnancy, cervical circlage was performed abdominally as an interval procedure. Since we considered her at very high risk of preterm delivery she was treated with Nimesulide, administered once daily as a 200 mg suppository from 16 weeks. Betamethasone 12 mg i.m. was given twice at 12 hourly intervals once every week from the 24th week until delivery. Ultrasound scans were performed at weekly intervals for measurement of amniotic fluid index (AFI) from 16 weeks and for ductal pulsatility index and peak velocity from 25 weeks. Doppler flow indices for the ductus and for the umbilical and middle cerebral arteries remained normal. Amniotic fluid index also remained normal throughout the pregnancy. There were no episodes of threatened preterm labour or contractions until Nimesulide was electively discontinued at 34 weeks. Seven days later she laboured and was delivered by caesarean section. The baby was ventilated for one day because of mild respiratory distress syndrome and then had an uncomplicated neonatal course.

Figure 6 shows the effect of prophylactic Nimesulide upon amniotic fluid index in a single patient at very high risk of preterm labour. Nimesulide was given from 17 to 34 weeks. Preterm labour began 7 days after therapy was stopped. Amniotic fluid index remained within normal limits throughout treatment whereas treatment with the COX non-selective drug Indomethacin characteristically causes oligohydramnios within 7 days.

CLAIMS

1. A method of substantially preventing or reducing at least one of the changes in the female reproductive system associated with the onset or continuation of labour the method comprising administering to the female an effective amount of a compound which selectively inhibits cyclo-oxygenase-2 (COX-2) function.

2. A method according to Claim 1 wherein pre-term labour is substantially prevented or reduced.

3. A method according to Claim 1 or 2 wherein uterine contractility, or uterine contractions, associated with pre-term labour are substantially prevented or reduced.

4. A method according to Claim 1 or 2 wherein ripening of the cervix is substantially prevented or reduced.

5. A method of substantially preventing or reducing uterine contractility, the method comprising administering to the female an effective amount of a compound which selectively inhibits COX-2 function.

6. A method according to Claim 5 wherein the female is pregnant and uterine contractions occur during pregnancy.

7. A method according to Claim 5 wherein the uterine contractions are associated with menorrhagia.

8. A method according to Claims 1 or 5 wherein the compound is

administered following (a) signs of pre-term labour or (b) an indication that the pregnant woman is at risk of pre-term labour or (c) symptom of menorrhagia.

9. A method according to any one of Claims 1 to 6 wherein the compound is administered during the second or third trimester of pregnancy.

10. A method according to Claim 9 wherein the compound is administered prophylactically.

11. A method according to any one of Claims 1 to 10 wherein the compound is administered intravaginally.

12. A method according to any one of the preceding claims wherein the compound selectively inhibits COX-2 enzyme activity.

13. A method according to any one of Claims 1 to 1 wherein the compound selectively inhibits COX-2 enzyme production.

14. A method according to any one of Claims 1 to 13 wherein the compound is any one of nimesulide, flosulide, or meloxicam.

15. A method according to Claim 9 wherein the female is administered between 0.1 and 40 mg/kg of nimesulide or flosulide.

16. A pharmaceutical composition comprising a compound which selectively inhibits COX-2 function and a further component that is usefully administered to a female who has or is at risk of pre-term labour, or a female who has or is at risk of uterine contractions, or

a female who suffers from menorrhagia.

17. A composition according to Claim 16 wherein the further compound is a progestogen or a progestin or other progestational agent.

18. A pharmaceutical composition comprising a compound which selectively inhibits COX-2 function wherein the composition in a form adapted for delivery to the female reproductive system.

19. A composition according to Claim 18 in a form adapted for delivery to the vagina or cervix.

20. A composition according to Claim 18 or 19 in the form of a pessary, gel or ring.

21. A composition according to Claim 18 or 19 in a form which is relatively stable to acidic conditions.

22. A composition according to Claim 18 in a form which is compatible with the amniotic fluid.

23. A composition according to Claim 22 comprising said compound and a further component which has substantially the same pH or osmotic tension as amniotic fluid.

24. A composition according to any one of Claims 16 to 23 wherein the compound selectively inhibits COX-2 enzyme activity.

25. A composition according to any one of Claims 16 to 23 wherein the

compound selectively inhibits COX-2 enzyme production.

26. A composition according to any one of Claims 16 to 23 wherein the compound is any one of nimesulide, flosulide, or meloxicam.

27. Use of a compound which selectively inhibits COX-2 function in the manufacture of a medicament for substantially preventing or reducing uterine contractility, or for substantially preventing or reducing at least one of the changes in the female reproductive system associated with the onset or continuation of labour.

28. Use of a compound which selectively inhibits COX-2 function as a tocolytic agent.

29. Any other novel use of a compound which selectively inhibits COX-2 function as herein described.

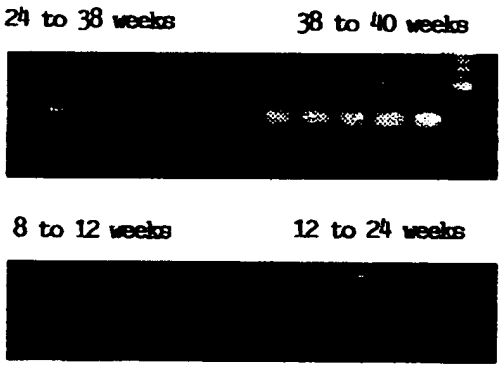


Figure 1

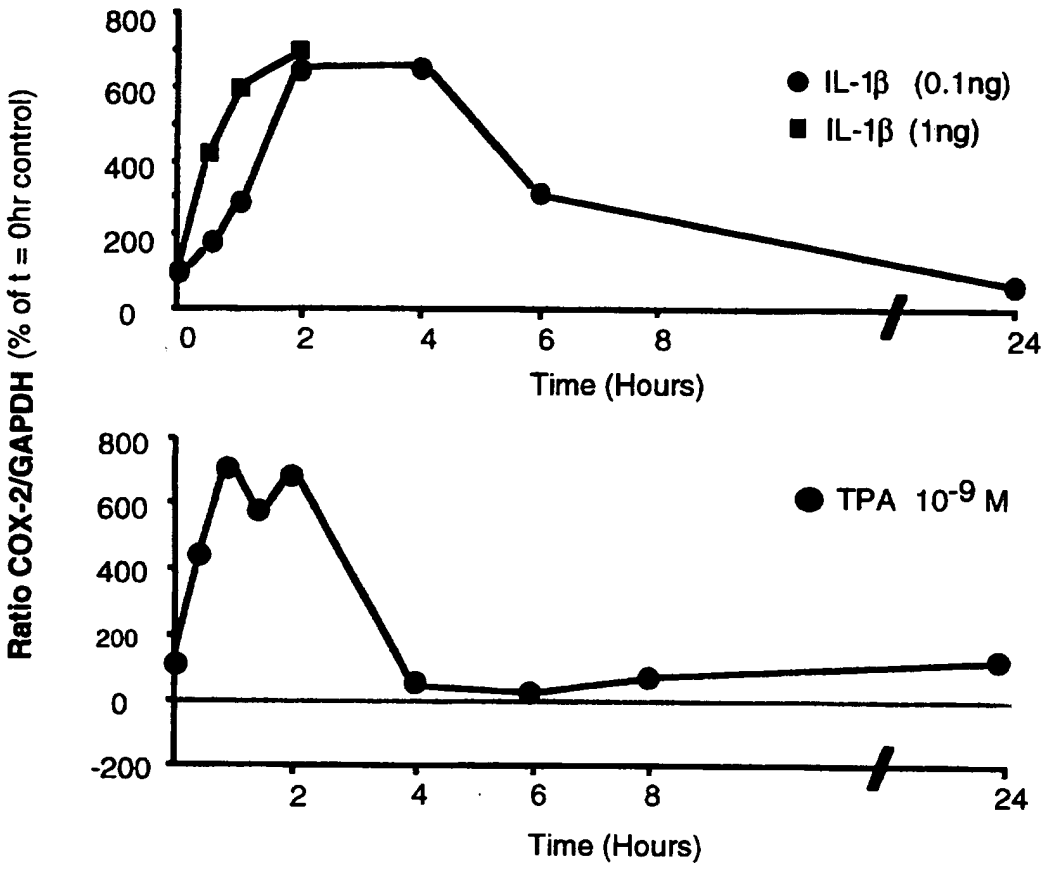

Ratio COX-2/GAPDH (% of t = 0hr control)
800
600
400
200
0
0
2
4
6
8
24
Time (Hours)
IL-1β (0.1ng)
IL-1β (1ng)
800
600
400
200
0
-200
2
4
6
8
24
Time (Hours)
TPA 10-9 M

Figure 2

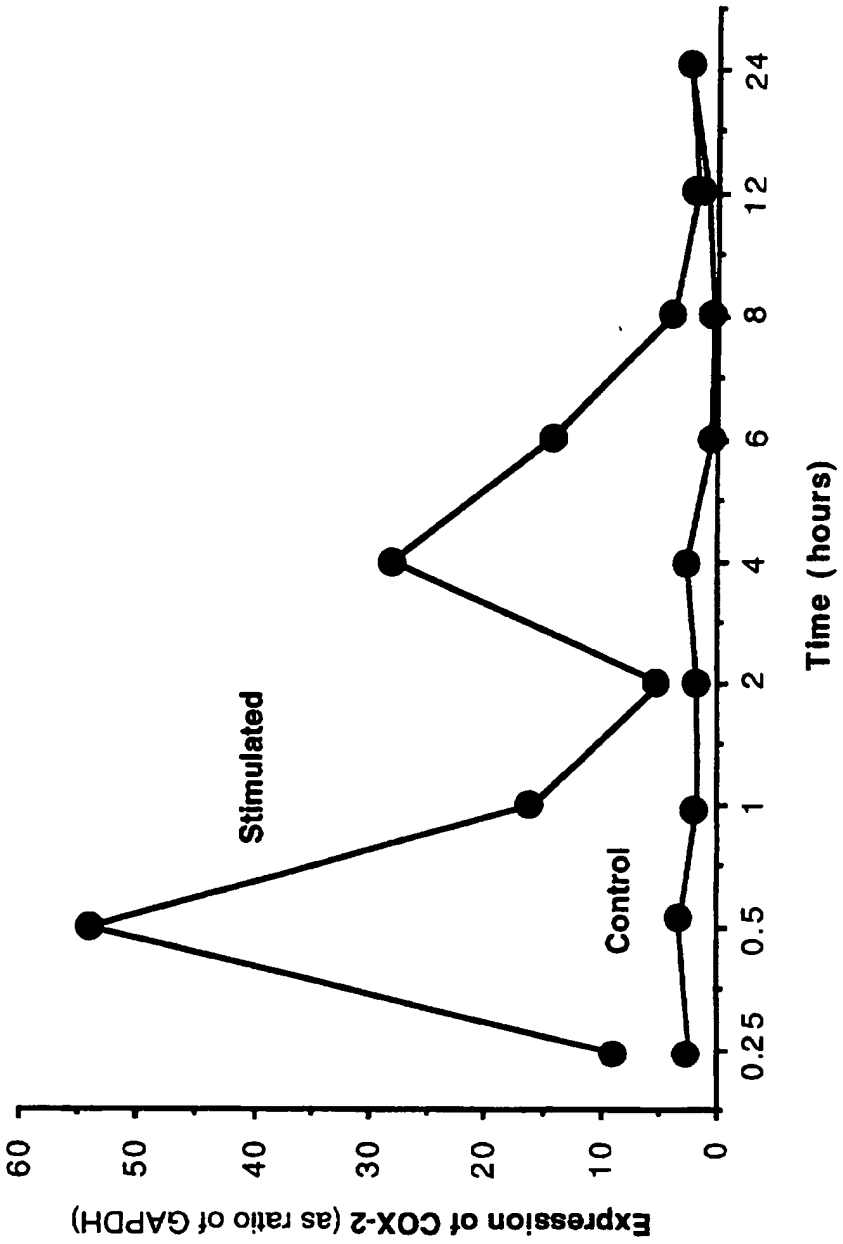

Expression of COX-2 (as ratio of GAPDH)
60
50
40
30
20
10
0
0.25
0.5
1
2
4
6
8
12
24
Time (hours)
Stimulated
Control

Figure 3

Figure 4

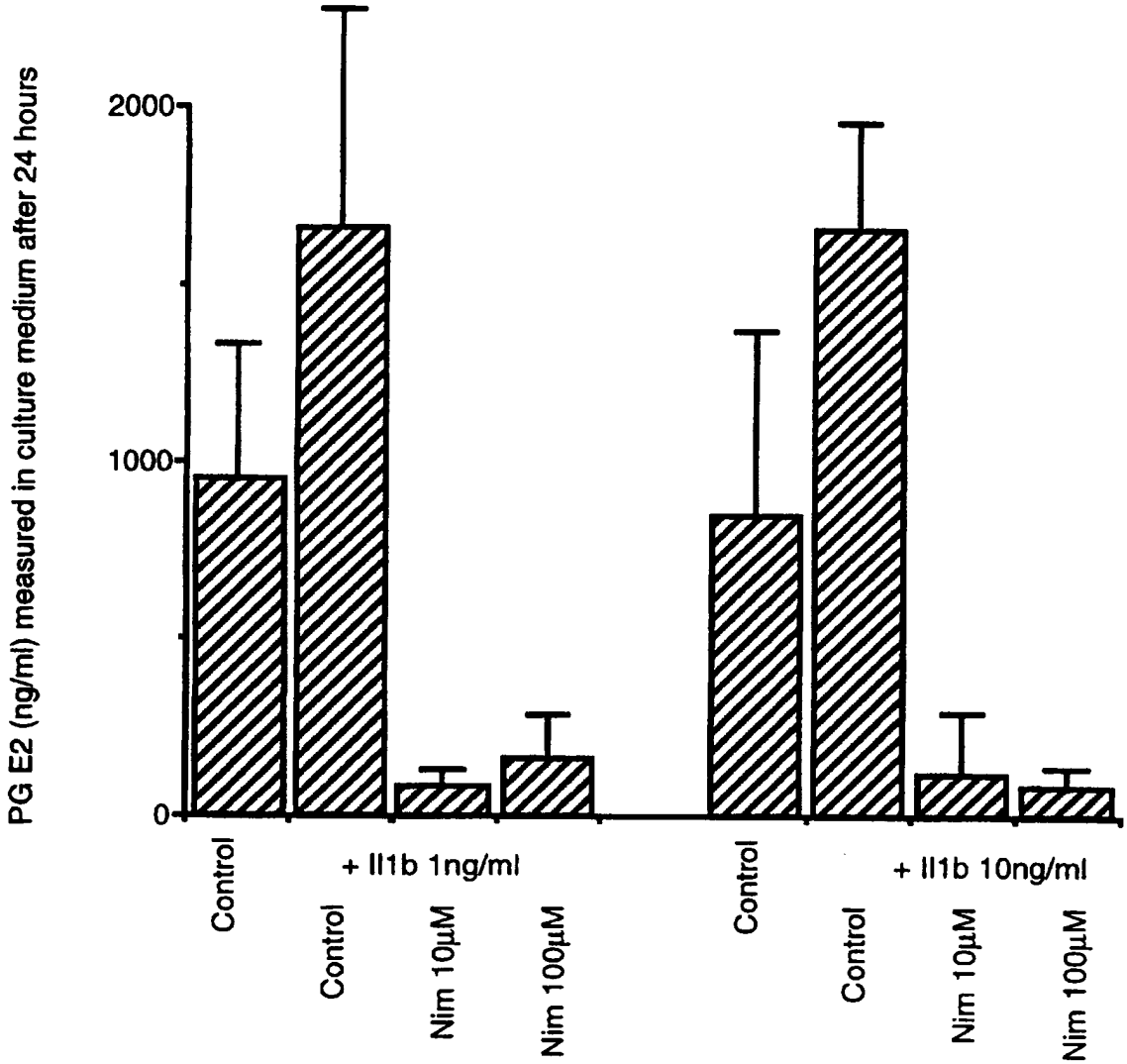

PG E2 (ng/ml) measured in culture medium after 24 hours
2000
1000
0
Control
+ Il1b 1ng/ml
Control
Nim 10µM
Nim 100µM
Control
+ Il1b 10ng/ml
Control
Nim 10µM
Nim 100µM

Figure 5

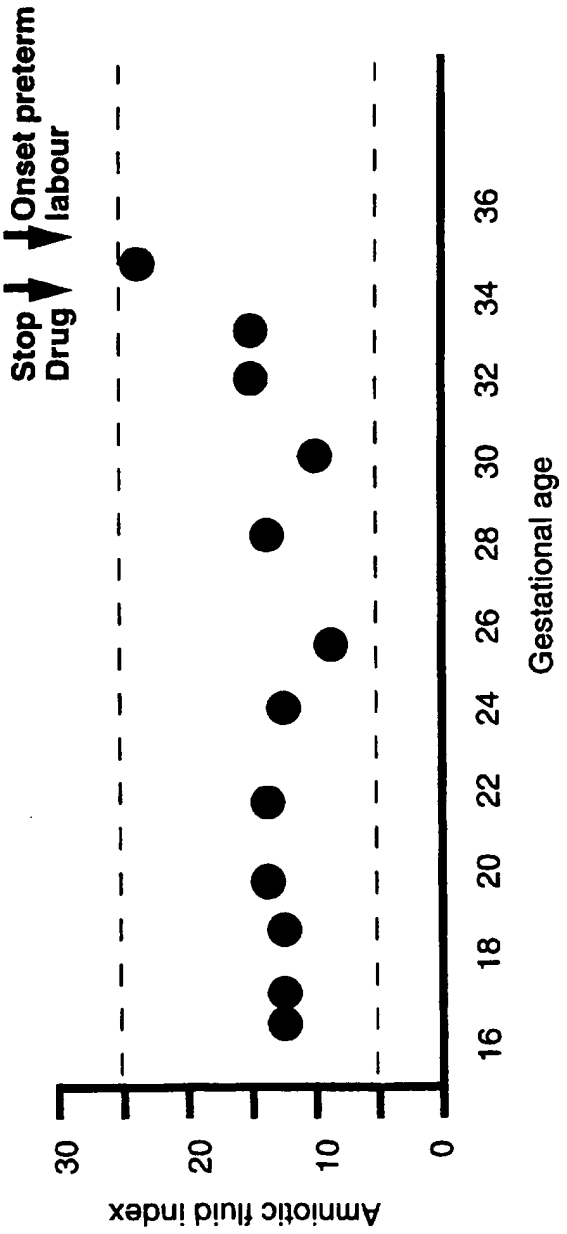

Stop Drug
Onset preterm labour
Amniotic fluid index
30
20
10
0
16
18
20
22
24
26
28
30
32
34
36
Gestational age

Figure 6

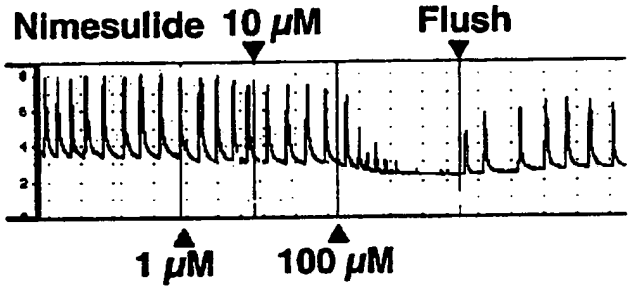


Figure 7

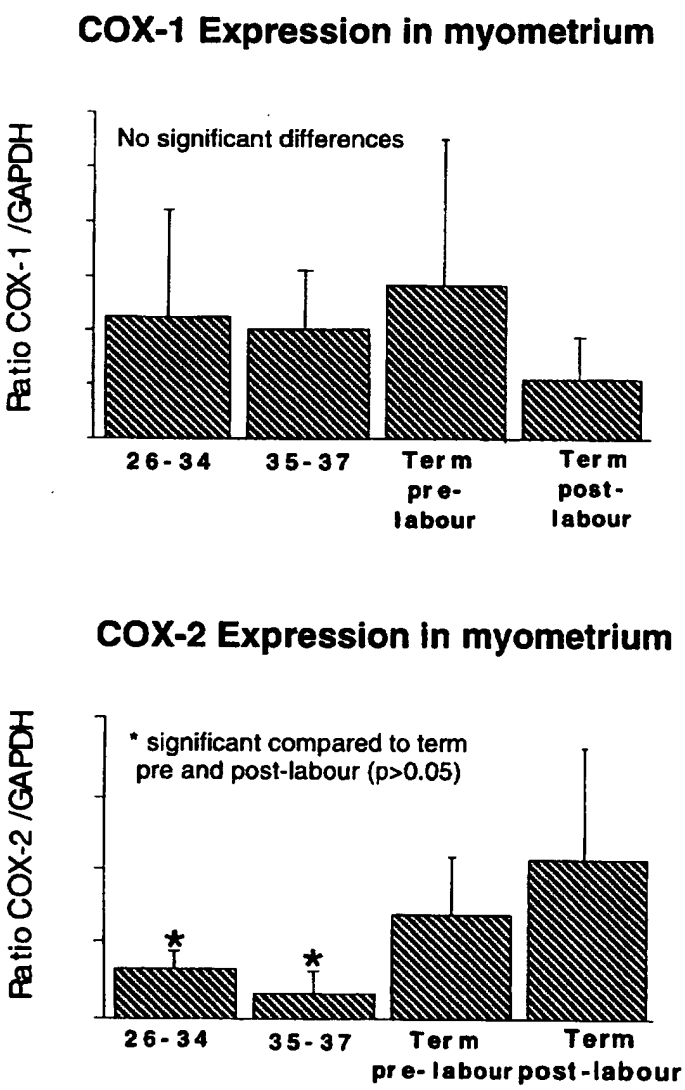

COX-1 Expression in myometrium
No significant differences
Ratio COX-1 /GAPDH
26-34
35-37
Term pre-labour
Term post-labour
COX-2 Expression in myometrium
* significant compared to term pre and post-labour (p>0.05)
Ratio COX-2 /GAPDH
26-34
35-37
Term pre-labour
Term post-labour

Figure 8

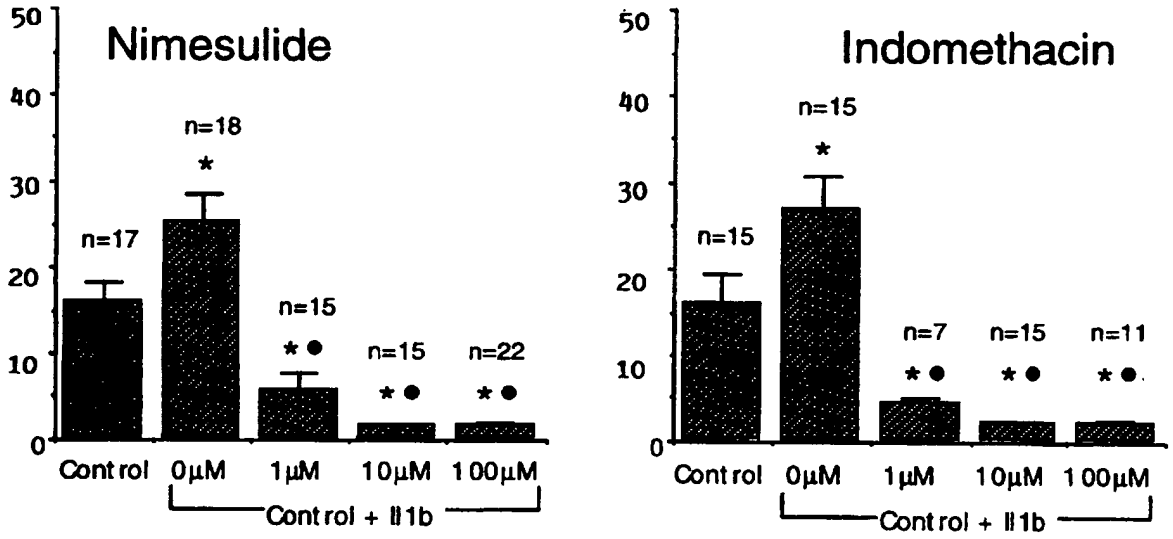

Nimesulide
50
40
30
20
10
0
n=17
n=18
*
n=15
* ●
n=15
* ●
n=22
* ●
Control
0μM
1μM
10μM
100μM
Control + Il1b
Indomethacin
n=15
n=15
*
n=7
* ●
n=15
* ●
n=11
* ●

Figure 9

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 97/00529

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/18 A61K31/63 A61K31/54 //(A61K31/18,A61K31:57, 31:565),(A61K31/63,A61K31:57,31:565),(A61K31/54,A61K31:57,31:565)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 14977 A (MERCK FROSST CANADA INC ;CROMLISH WANDA A (CA); KENNEDY BRIAN P (C) 7 July 1994 see page 1, line 23 - line 35 see page 2, line 1 see page 19; table II see page 22, line 32 - line 35 ---	1,3,5, 12,14, 18,24, 26,27,29
X	ARCH. INT. PHARMACODYN. THER., vol. 238, no. 2, 1979, pages 233-243, XP000674552 MALOFIEJEW ET AL.: "Influence of R-805 on the contractility and reactivity of rat endometrium" see the whole document --- -/--	5,12,14, 27,29

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
17 June 1997	03.07.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Gac, G

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DRUGS, vol. 46, no. suppl, 1993, pages 129-133, XP000674525 PULKKINEN ET AL.: "Nimesulide in dysmenorrhoea" see the whole document ---	5,12,14, 15,27,29
X	J. CLIN. PHARMACOL., vol. 27, no. 1, January 1987, pages 67-69, XP000564564 PULKKINEN ET AL.: "Alterations in intrauterine presseure, menstrual fluid prostaglandin F levels, and pain in dysmenorrheic women treated with nimesulide" see the whole document	5,12,14, 27,29
Y	---	7,8
Y	CAN. J. PHYSIOL. PHARMACOL., vol. 65, 1987, pages 2'81-2084, XP000674686 TSANG ET AL.: "Endometrial prostaglandins and menorrhagia: influence of a prostaglandin synthetase inhibitor in vivo" see the whole document ---	7,8
P,X	DRUG NEWS PERSPECTIVES, vol. 9, no. 2, March 1996, pages 123-128, XP000674518 BOTTING ET AL.: "Further developments in the nature of the induction of COX-2 in pathological and physiological conditions and selective inhibitors of this enzyme were presented at the Williams Harvey conference held in London : COX-2 : selective inhibitors in clinical trials" see page 125 - page 127, left-hand column	1-3,8,9, 12-15, 27-29
A	---	16,23,26
P,X	WO 96 41645 A (SEARLE & CO) 27 December 1996 see page 3 - page 5 see page 6, line 25 see page 7, line 29 ---	5,12,14, 16,18, 24,26, 27,29
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PROSTAGLANDINS, Vol. 52, no 3, September 1996, pages 199-208, ISHIHARA et al. : "Prostaglandin E2 production by endogenous secretion of Interleukin-1 in decidual cells obtained before and after labor" XP002033179 see the whole document	1-3,5,6, 8,9,13, 18,25, 27-29
P,X	WO 96 28145 A (SCHERING AKTIENGESELLSCHFT) 19 September 1996 see page 4 - page 6	16-20, 24-26
Y	PROSTAGLANDINS, vol. 47, Jan. 1994, pages 55-59, FUTAKI et al.: "NS-398, a new anti-inflammatory agent selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity in vitro" XP002033186 see the whole document	1-3,5,6, 9,12,13, 16,17, 27-29
Y	PROSTAGLANDINS, vol. 49, Jan. 1995, pages 15-26 ISHIHARA et al.: "Interleukin-1-beta-stimulated PGE2 production from early first trimester human decidual cells is inhibited by dexamethasone and progesterone XP002033187 see the whole document	1-3,5,6, 9,12,13, 16,17, 27-29
Y	J. CLIN. ENDOCRINOL. METABOLISM, vol. 78, no. 6, June 1994, pages 1396-1402, XP000674683 TEIXEIRA ET AL.: "Prostaglyndin endoperoxide-H synthase (PGHS) activity and immunoreactive PGHS-1 and PGHS-2 levels in human amnion throughout gestation, at term, and during labor" see the whole document	1-3,5,6, 9,27
A	CLIN. PHARMACY, vol. 2, no. 1, January 1983 - February 1983, pages 29-44, XP000674519 SOUNEY ET AL.: "Pharmacotherapy of preterm labor" see the whole document	1-29

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ACTA BIOL. MED. GER. , vol. 37, no. 5-6, 1978, pages 923-930, XP000674553 WIQUIST ET AL.: "Treatment of premature labor by prostaglandin synthetase inhibitors" see the whole document	1-6,8,9, 16,17
A	CLIN. OBST. GYAECOL., vol. 8, no. 2, August 1981, pages 507-520, XP000674548 CALDER ET AL.: "Oxytocics and tocolytics" see page 510 - page 512 see page 514 see page 517	1-6,8,9, 16
A	PROSTAGLANDINS, vol. 39, no. 5, May 1990, pages 515-523, XP002033185 CABROL ET AL.: "Cyclooxygenase and lipoxygenase inhibitors induced changes in the distribution of glycosaminoglycans in the pregnant rat uterine cervix" see the whole document	1,4,9,12
A	ACTA OBST. GYN. SCAND., vol. 113, no. suppl., 1983, pages 71-76, XP000674545 FORMAN ET AL.: "Aspects of inhibition of myometrial hyperactivity in primary dysmenorrhea" see the whole document	16
A	PROSTAGLANDINS, vol. 30, no. 1, July 1985, pages 13-20, XP002032991 COHEN ET AL.: "Prostaglandin biosynthesis stimulatory and inhibitory substances in human amniotic fluid during pregnancy and labor" see the whole document	1,2,5,6, 8-13, 16-25
A	CAN. J. SURG., vol. 29, no. 5, September 1986, pages 305-307, XP000674677 BOYD ET AL.: "Dysfunctional uterine bleeding " see the whole document	5,7,8, 16,17

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LANCET, vol. 1, no. 7963, 10 April 1976, pages 774-776, XP000674678 ANDERSON ET AL.: "Reduction of menstrual blood loss by prostaglandin-synthetase inhibitors" see the whole document ---	5,7,8
A	PROC. NATL ACAD. SCI., vol. 90, December 1994, pages 11693-11697, XP002032992 MITCHELL ET AL.: "Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase" see page 11695; table 2 see page 11696, right-hand column see abstract -----	1,29

INTERNATIONAL SEARCH REPORT

Int· ·ional application No.
PCT/GB 97/00529

B x I . Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 1-15
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte nal Application No
PCT/GB 97/00529

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9414977 A	07-07-94	US 5543297 A	06-08-96
		AU 5690694 A	19-07-94
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		EP 0675964 A	11-10-95
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